28 and 37 being the independent claims. The amendment is being made to put the claims in better form for consideration upon appeal. These changes are believed to introduce no new matter, and their entry is respectfully requested.

Based on the above amendment and the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

***Rejection under 35 U.S.C. § 112***

The Examiner has rejected claims 1-21 under 35 U.S.C. § 112, first paragraph, on the grounds that the application allegedly does not enable the full scope of the claims. Applicants respectfully traverse this ground for rejection. In summary, the Examiner contends that the application "does not reasonably provide enablement for a method of treating any and all forms of primary or secondary hypertension in all mammals comprising introducing by any route of delivery, any vector encoding any nitric oxide synthase gene under the control of any promoter." (Paper No. 11, at p. 3).

According to the Examiner a claim directed to "a method of inducing vasodilation in a mammal comprising: introducing into the lungs of a mammalian patient in need of pulmonary vasodilation an aerosolized adenoviral vector comprising a wild type nitric oxide synthase gene operably linked to the CMV promoter, wherein the introduction of said gene into the lungs of said patient results in pulmonary vasodilation that does not significantly affect systemic blood pressure or cardiac index" is enabled. (Paper No. 11, at p. 2). In addition, the Examiner indicates that a claim directed to a method of treating hypoxic pulmonary hypertension in rats using the same vector, wherein this treatment does not significantly affect systemic blood

pressure or cardiac index is enabled. (Paper No. 11, at p. 2-3). In the interest of expediting prosecution, Applicants have amended the method claims to recite "mammal" instead of patient, and by adding "wherein said treatment does not significantly affect systemic blood pressure or cardiac index." Support for this amendment can be found in the specification, *inter alia*, at page 9, lines 20-23, page 11, line 16, page 15, lines 22 and 30, page 6, lines 25-27, and original claims 7 and 16.

A claimed invention is enabled if one skilled in the art could make or use the invention, relying on information disclosed in the specification coupled with information known in the art, without undue experimentation. *In re Wands*, 8 U.S.P.Q.2d 1400, 1404 (Fed. Cir. 1988); see also MPEP § 2164.01 (7th ed. July, 1998). In addition, an Applicant need not teach how to make and use every embodiment within a claim, rather, 35 U.S.C. § 112, first paragraph, requires that "the scope of the claims must bear a reasonable correlation to the scope of enablement provided by the specification to persons of ordinary skill in the art." *In re Fisher*, 427 F.2d 833, 839, 166 U.S.P.Q. 18, 24 (C.C.P.A. 1970); *see also* MPEP § 2164.08 (7th ed. July 1998).

Several arguments have been raised in support of the enablement rejection. First, the Examiner contends that the specification fails to enable addition or deletion mutants of any NOS gene. Information known in the art as of Applicants' priority date, and that provided by the specification provided ample guidance for the selection of appropriate amino acid substitutions, deletions, or additions that would result in a NOS mutant with the activity of the wild type for that isoform, and a means for testing NOS mutants for the activity required to induce pulmonary vasodilation or treat pulmonary hypertension. The specification provides this guidance, *inter alia*, at page 12, line 19, to page 14, line 7, which teaches, for example,

conservative substitutions and examples of preferred mutations, and at page 11, lines 19-23, which discloses regions of shared homology between ceNOS and nNOS, and thereby provides guidance as to where mutations would be most likely to disrupt activity (and would therefore be disfavored). Finally, the specification teaches *inter alia*, in Examples 6 and 7, the specific activity of NOS required to produce sufficient NO to result in pulmonary vasodilation and to treat pulmonary hypertension, and a method of testing for the appropriate activity.

In addition to the information provided in the specification, much information was known in the art regarding NOS mutants. For example, several articles disclosed mutations affecting wild type activity, some of which we describe here. Robinson and Michel discloses that substitution of two cysteine residues in ceNOS with serine residues completely eliminates palmitoylation, a covalent modification process which plays a role in ceNOS membrane association. Robinson, L.J. and Michel, T., *Proc. Nat. Acad. Sci. USA 92:*11776-11780 (1995) (Attached as Exhibit A). McMillan and Masters, and Richards and Marletta each disclose that a mutation in Cys-415 in rat nNOS abolishes the binding of heme to the enzyme, a critical step in the dimerization of the enzyme that is necessary for catalytic activity. McMillan, K. and Masters, B.S.S., *Biochemistry 34:*3686-3693 (1995) (Attached as Exhibit B); Richards, M.K. and Marletta, M.A., *Biochemistry 33:*14723-14732 (1994)(Attached as Exhibit C). Xie *et al.* (1994), discloses that mutation of residues 1121-1144 of mouse iNOS results in abolition of NADPH binding and enzymatic activity. Xie, Q.-w. *et al.*, *J. Biol. Chem. 269:*28500-28505 (1994) (Attached as Exhibit D). Cho *et al.*, discloses that mutations in Gly-450 and Ala-453 of mouse iNOS eliminates catalytic activity. Cho, H.J. *et al.*, *Proc. Nat. Acad. Sci. USA 92:*11514-11518 (1995)(Attached as Exhibit E). Xie *et al.*(1996), discloses that a mutation in Cys-194 of mouse iNOS eliminates heme binding and consequently,

enzymatic activity. Xie, Q.-w. *et al.*, *Proc. Nat. Acad. Sci. USA 93:*4891-4896 (1996)(Attached as Exhibit F). Ogura *et al.*, discloses that amino acid residues 504-608 of rat nNOS, which make up the $NH_2$-terminal catalytic domain, are critical for enzyme activity. Ogura, T. *et al.*, *Biochem. Biophys. Res. Commun. 193:*1014-1022 (1993) (Attached as Exhibit G). Finally, Wang and Marsden, provides general structure-function information about the NOS isoforms. Wang, Y., and Marsden, P.A., "Nitric Oxide Synthases: Gene Structure and Regulation," in: *Nitric Oxide, Biochemistry, Molecular Biology, and Therapeutic Implications*, Ignarro, L., eds., (Academic Press 1995) (Attached as Exhibit H).

The information provided by the specification in combination with information known in the art provided guidance regarding the selection of mutations that would not result in the loss of activity. Using this information, one skilled in the art would be able to make and use NOS mutants, avoiding the critical regions described by the specification and which were known in the art, and then test for NOS activity as described by the specification, without undue experimentation. Thus, NOS mutants within the full scope of the claims are enabled.

The Examiner's next argument is that the claims are not enabled for any and all vector-promoter combinations. However, Applicants need not enable the use of any and all vector-promoter combinations, only those required by the claims, which call for the introduction of a NOS gene into the lung. Moreover, the scope of the claims need only bear a reasonable correlation to what has been enabled by the specification to persons of skill in the art. *Fisher*, 427 F.2d at 839, 166 U.S.P.Q. at 24; *see also* MPEP § 2164.08. Applicants assert that the selection of vectors that could be used to transduce lung tissue, and expression elements for expression in this tissue, was within the knowledge of one of skill in the art by Applicants' priority date.

First, those of skill in the art were aware, as of Applicants' priority date, of the limitations on transferring genes into slowly or nondividing cells, like some of the cell types found in lung tissue. *See* Naldini *et al.*, *Science 272:* 263-67, at p.263, col. 1, para. 1 (April 1996) (Attached as Exhibit I). Several expression systems for transducing these types of cells, in addition to adenoviral vectors, were known in the art by Applicants' priority date. For example, Naldini *et al.* discloses the use of a HIV-based lentiviral vector to mediate stable *in vivo* gene transfer into nondividing cells. Naldini *et al.*, *Science* 272:263-267 (1996). Flotte *et al.* discloses the use of a recombinant adeno-associated virus (rAAV) as a vector for transducing rabbit lung epithelium with the cystic fibrosis transmembrane conductance regulator. Flotte, T.R. *et al.*, *J. Biol. Chem.* 268:3781-3790 (1993) (Attached as Exhibit J).

Moreover, viral vectors are not the only means available for delivering genes into cells found in the lung. Liposomes, for example, were also known as delivery vehicles for genes by Applicants' priority date. Liposome gene delivery to the pulmonary vasculature *in vivo* is disclosed in Muller, D.W.M., *et al.*, *Circ. Res. 75:* 1039-49 (1994)(attached as Exhibit K). In addition, Kreuzer *et al.* reports that the use of adenovirus to assist lipofection resulted in efficient in vitro gene transfer of a transgene to human smooth muscle cells in the pulmonary vasculature. Kreuzer, J. *et al.*, *Atherosclerosis 124:*49-60 (July 1996)(Attached as Exhibit L). According to this paper, adenoviral-assisted lipofection increased transfer 1000-fold, compared to lipofection alone. *Id.* at 53.

Various expression control elements for the expression of transferred genes in lung tissue, in addition to those disclosed in the specification, were also known. For example, Huffman *et al.*, discloses the use of the promoter of the human surfactant protein-C (SP-C) gene to construct a chimeric gene that directed expression in the respiratory epithelium of

mice. Huffman, J.A. *et al.*, *J. Clin. Invest.* 97(3):649-655 (February 1996) (Attached as Exhibit M). Maniatis *et al.*, discloses the use of the SV40 early gene enhancer and the enhancer/promoter combination derived from the long terminal repeat (LTR) of the Rous sarcoma virus, both of which are active in a wide range of cell types. Maniatis *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd ed. (Cold Spring Harbor Laboratory Press 1989) (Attached as Exhibit N).

These articles demonstrate that, as of Applicants' priority date, one skilled in the art, relying on information disclosed in the specification and known in the art, would be able to make and use gene transfer vectors and expression control elements within the full scope of the claims, without undue experimentation.

The Examiner also maintains that the claims are not enabled for any and all routes of vector delivery. Applicants need not enable the use of any and all methods of delivery, only those required by the claims, which call for the introduction of a NOS gene into the lung. Moreover, the scope of the claims need only bear a reasonable correlation to what has been enabled by the specification to persons of skill in the art. *Fisher*, 427 F.2d at 839, 166 U.S.P.Q. at 24; *see also* MPEP § 2164.08.

Several routes of gene delivery, in addition to aerosol delivery, are disclosed in the specification, and were known by Applicants' priority date, for delivering genes to lung tissue. The specification discloses several methods of delivery, including intratracheal delivery, intravenous delivery, intraarterial delivery, and *ex vivo* transfection of cells with the introduction of those cells into the lung. *See* Specification at page 16, lines 14-28.

In addition methods of gene delivery to the lung were known in the art by Applicants' priority date. For example, the intraarterial and intravenous administration of liposomes

carrying genes to the pulmonary vasculature were known. Liposome gene delivery to the pulmonary vasculature *in vivo* by percutaneous catheterization of the right femoral vein is disclosed in Muller *et al.*, *Circ. Res. 75:* 1039-49 (1994); *see also* Conary, J.T. *et al.* J. Clin. Invest. 93:1834-1840 (1994)(attached as Exhibit O). Liposomal gene delivery by transcatheter injection into the right posterior basal pulmonary artery of a human patient is disclosed in Nabel *et al.*, *Human Gene Ther. 5:*1089-94 (1994)(attached as Exhibit P). In addition, various *ex vivo* gene delivery methods for transducing endothelial and smooth muscle cells and reintroducing them into a mammal were also known. Nabel, *et al.*, provides a brief review of these methods. Nabel, E.G., *Cardiovasc. Res. 28:*445-55, at 450 (1994)(Attached as Exhibit Q).

Thus, as of Applicants' priority date, one skilled in the art, relying on information disclosed in the specification and known in the art, could introduce a NOS gene into the lung within the full scope of the claims without undue experimentation.

The Examiner relies on Verma, I.M. and Somia, N., *Nature 389:*239-242 (1997), and on Orkin, S.H. and Motulsky, A.G., "Report and Recommendations of the Panel to Assess the NIH Investment in Research on Gene Therapy" (Dec. 7, 1995), for the proposition that gene therapy is unpredictable, to support her rejection. Specifically, the Examiner has relied on these articles for the proposition that the art recognized several problems associated with gene therapy, including "problems associated with the vector systems available at the time of filing: the inability of retroviral vectors to infect non-dividing cells such as those found in the lung, the lack of stable transgene expression due to immunological recognition of virally infected cells, the lack of sufficient levels of gene expression due to problems associated with the use of any and all promoters such as inducible promoters and cell-specific promoters, and the

unpredictability of achieving targeted gene delivery where the vector is introduced at a location other than the target location." (Paper No. 11, at p. 5).

Applicants addressed above the issues of targeted gene delivery, selecting vectors for transducing nondividing or slowly dividing cells, and selecting expression control elements for controlling gene expression in lung tissue. With regard to the lack of stable transgene expression due to immunological recognition of virally infected cells, the specification addresses this issue, *inter alia*, at page 17, lines 1-4, where it discloses that immunosuppressive agents may be used to lengthen the clearance time of the vector from the patient.

The Examiner's next contention is that the claims drawn to a pharmaceutical composition comprising a NOS gene and an immunosuppressive agent are not enabled because cyclosporin is one of the immunosuppressive agents explicitly named in the application, and cyclosporin A has been reported in Roullet *et al.*, *J. Clin. Invest. 93:*2244-50 (1993), to increase systemic blood pressure when administered.

Applicants wish to note that the observations made in Roullet *et al.* in *J. Clin. Invest. 93:*2244-50 (1993), were based on data from experiments with mesenteric arteries of Sprague Dawley rats, while Applicants' data are based on experiments with Wistar rats. Roullet *et al.* recognize that the data on the effect of cyclosporin A on blood pressure previously reported are inconsistent, perhaps due to differences in rat strain between studies. Roullet, J.-B., *et al.*, *J. Clin Invest. 93:*2244-50, at 2246, col. 1, lines 26-29 (1993). Moreover, pulmonary and systemic (e.g., mesenteric) vessels are known to significantly differ in vasoreactivity to various stimuli. In response to hypoxia, pulmonary vessels constrict whereas systemic vessels dilate.

Therefore, extrapolating data observed in mesenteric vessels to pulmonary vessels should be done with extreme caution as different physiological mechanisms apply in these systems.

In addition, cyclosporin A is only one immunosuppressive agent that falls within the scope of the claim. Several immunosuppressive agents were known in the art at the time of Applicants' priority date. *See, e.g.*, *Remington's Pharmaceutical Sciences*, 1142-62, Gennaro, A.R., ed., Mack Pub. Co. (18th Ed. 1990)(Attached as Exhibit R). It would not require undue experimentation to test the effect of other known immunosuppressive agents on systemic blood pressure, for those agents for which this information was not already known. However, although Applicants disagree with the contention that claims drawn to a pharmaceutical composition comprising a NOS gene and an immunosuppressive agent are not enabled because of the report in Roullet *et al.*, Applicants have canceled claim 18 in the interest of expediting prosecution.

Applicants have amended the claims to add claim 38, which corresponds to canceled claim 18. Claim 38 recites a method of inducing pulmonary vasodilation comprising: administering to a mammal in need of pulmonary vasodilation (1) an effective amount of a pharmaceutical composition comprising a nucleic acid encoding a nitric oxide synthase operably linked to an expression control element and a means for transducing said nucleic acid into pulmonary tissue, and (2) an effective amount of at least one drug selected from the group consisting of an immunosuppressive agent and a phosphodiesterase inhibitor, wherein said treatment does not significantly affect systemic blood pressure or cardiac index. Applicants have replaced claim 18 with claim 38 in order to include the administration of the claimed pharmaceutical composition comprising a NOS gene, when an immunosuppressive agent or phosphodiesterase inhibitor is administered in a composition separate from the composition

comprising the NOS gene. Support for this claim can be found in the specification, *inter alia*, at page 17, lines 1-6.

New claim 38 requires that the administration of the NOS gene, and the effective amount of a drug selected from an immunosuppressive agent or a phosphodiesterase inhibitor, not significantly affect systemic blood pressure or cardiac index. Thus, to practice within this claim, one of ordinary skill in the art would have to select an immunosuppressive agent that does not have, for example, a significant effect on systemic hypertension. As discussed above, it would not have required undue experimentation for one skilled in the art to determine which agents may be used in the claimed method, for those agents for which information regarding the effect on systemic blood pressure or cardiac index was not already known. Therefore, the claimed method is enabled.

The Examiner's last contention is that the claims are not enabled for the treatment of any and all forms of pulmonary hypertension in any and all mammals. In support of this contention, the Examiner argues that the rat model, used in the examples in the application, is not a good model for pulmonary hypertension in humans, therefore, the treatment of this disease is not enabled for humans. The Examiner relies on a statement in Heath that alleges that the rat is not a good model for this disease in humans, because the rat does not undergo the migration of smooth muscle cells into the intima of blood vessels, which occurs in the disease in humans. Heath, D., *Eur. Respir. Rev. 3:* 555-58 (1993). Further, the Examiner found Applicants' reliance on Roberts *et al.*,*Circ. Res. 76:*215-222 (1995), unpersuasive because the rat model was used for the experiment reported in that paper.

At most, the statement in Heath indicates that results from tests showing the effect of nitric oxide on vascular structural changes in rats may not be used to draw conclusion

regarding its effect on smooth muscle cell migration in humans. However, Rabinovitch et al., contradicts this statement in Heath, by teaching that one of three abnormal features found in arterial circulation in the lungs of hypoxic rats is the "extension of muscle [i.e., migration of muscle cells] into smaller and more peripheral arteries than normal." Rabinovitch, M., *et al.*, *Am. J. Physiol. 236:*H818-27, at 822, col. 1, lines 29-32 (1979)(Attached as Exhibit S); *see also* Reid, L and Meyrick, B., *Excerpta Medica, Metabolic Activities of the Lung, Ciba Found. Symp. 78:*37-60, at 44, para. 4 to 45, para. 1 (1980)(Attached as Exhibit T); Roberts, J.D., *et al. Circ. Res. 76:* 215-222, at 215, col. 2, lines 22-26 (1995) (Attached as Exhibit U). Further, Rabinovitch *et al.*, states that the degree of abnormal extension (i.e., migration) of muscle into peripheral arteries "proved to convey the totality of morphological information: when abnormal extension of muscle is taken into account, neither increased wall thickness of the muscular arteries nor increased ratio of alveoli to arteries [the other two morphological changes reported to occur in the arteries of hypoxic rats] correlates with pulmonary artery pressure . . . . These two features are informative only as surrogates for extension of muscle." *Id.* at H824, col. 1, lines 1-12. In fact, nitric oxide inhalation was reported by Roberts *et al.*, to reduce the extension of smooth muscle into peripheral lung arteries. Roberts, J.D., *et al. Circ. Res. 76:* 215-222, at 215, col. 2, lines 22-26 (1995). These papers counter the assertion in Heath that the rat is not an appropriate model for pulmonary hypertension in humans.

In addition, the rat exhibits other structural changes in the pulmonary vasculature, including smooth muscle cell proliferation, differentiation and hypertrophy, that occur in pulmonary hypertension in humans. *See* Janssens, *et al.*, *J. Appl. Physiol.* 77(3):1101-1107 (1994) (Attached as Exhibit V); Roberts, J.D., *et al. Circ. Res. 76:* 215-222 (1995). These shared structural changes further support the use of the rat as a model for pulmonary

hypertension in humans. Moreover, Applicants' own post-filing experiments suggest that nitric oxide inhibits at least some of these structural changes to pulmonary vasculature. This data shows that the transfer of a NOS gene to the lung suppressed the muscular hypertrophy that accompanies hypoxia in rats.

Finally, the recitation of the word "treating" in the claims relating to the treatment of pulmonary hypertension does not require that the disease be cured. The specification defines "treating" on page 10, lines 10 through 13, as including "the administration of therapeutic compositions of the invention to a subject for purposes which may include prophylaxis, amelioration, prevention, or cure of a medical disorder, such as pulmonary hypertension." Thus, even if the administration of nitric oxide is never shown to prevent or alter the pathological structural changes of pulmonary hypertension in humans, the fact that it ameliorates the disease in humans is sufficient to enable these claims for the treatment of humans. Therefore, Applicants maintain that the claims drawn to methods of treating pulmonary hypertension are enabled.

## *Conclusion*

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. It is believed that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment is respectfully requested.

Respectfully submitted,

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